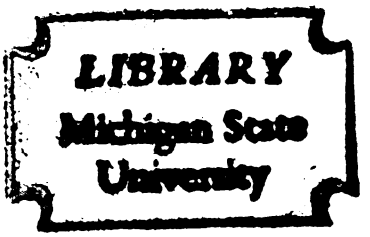


This is to certify that the

thesis entitled

INTERSPECIFIC AND INTERGENERIC
HYBRIDS WITH HORDEUM vulgare L.

presented by

James L. Nelson

has been accepted towards fulfillment
of the requirements for

M.S. degree in Crop & Soil Sciences

Major professor

Date 7/16/80

INTERSPECIFIC AND INTERGENERIC HYBRIDS WITH *HORDEUM vulgare* L.

By

James L. Nelson

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Crop and Soil Sciences

1980

G 116/86

ABSTRACT

INTERSPECIFIC AND INTERGENERIC
HYBRIDS WITH HORDEUM vulgare L.

By

James L. Nelson

Exhaustion of genetic variability within Hordeum vulgare for several important agronomic characteristics has necessitated interspecific and intergeneric hybridization of barley with its wild relatives. The continuing attempt to transfer desirable genes involves four hybrids with barley: H. jubatum, Agropyron trachycaulum, the hybrid (H. bogdanii x Elymus canadensis) and Hordelymus europeaus. Self and backcross infertility appear to be the primary impediments to successful introgression. The utilization of somatic recombination facilitated through both chemical treatment and gamma-irradiation, coupled with the production of H. vulgare haploid sectors, appears to be a possible solution to sexual incompatability. Screening of the doubled haploids for germinability in varying concentrations of NaCl is reported and the results indicate possible genotypic differences between the lines. A diallel cross between surviving seedlings at the highest concentration of NaCl (20,000 ppm) is proposed as an additional means of determining genotypic differences between the haploid-derived lines.

ACKNOWLEDGMENTS

I appreciate the assistance provided to me by the members of my committee: Dr. Everett Everson, Dr. David Smith, Dr. William Tai and Dr. Peter Carlson. Additionally, I wish to thank Dr. Carter Harrison for his editorial assistance.

Most of all I wish to acknowledge my deep appreciation for the late Dr. John E. Grafius, my major professor and friend. His vision, however poorly reflected in this research, was and is an inspiration for all scientists.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The object of the research reported in this thesis is the transfer of agronomically desirable traits to barley (Hordeum vulgare, L.). The traits or characteristics of primary importance are those for winterhardiness, salt tolerance, and resistance to cereal leaf beetle, although other traits are not without importance. For the traits mentioned above intraspecific variability for further improvement within H. vulgare is either absent or of low intensity. Thus, the need to resort to interspecific and intergeneric hybridization has become critical. The hybridization and consequent work reported in this thesis involves four types of wide hybrids, all with barley: H. jubatum, Agropyron trachycaulum, the hybrid (H. bogdanii x Elymus canadensis) and Hordelymus europeaus.

LITERATURE REVIEW

In the vast literature of wide hybridization, barley ranks low in terms of citations. Of the reported attempts to produce fertile offspring, few succeeded and of those crosses in which success has been reported actual gene transfers of any agronomic significance has been minimal. Before addressing these miniscule successes, a brief explanation of the difficulties is in order.

Of the major cereals grown as crops, only barley and rye are diploid; all others are polyploids. Within the polyploid species tremendous variability can be found which results directly from their polyploidy and by virtue of their numerous and sexually compatible wild relatives. The most notable of these polyploid cereals is allohexaploid wheat. Polyploidy also confers what Harlan (1966) has termed as "buffering" upon these species. Extremely wide crosses are tolerated both physiologically and genomically due to a multiplicity of functional, necessary enzymes and enzyme systems. Another form of buffering occurs via a high degree of heteozygosity which is best exemplified in corn, a highly outcrossed species. Any species which is buffered by either means is more amenable to wide hybridization and introgression.

Barley is at the other end of the spectrum. It is a diploid (2n=2x=14), and it is the most autogamous of all the cereals. Thus it has neither of the buffering mechanisms detailed by Harlan. Furthermore, barley has no closely related species within its genus. Exclude from consideration the three wild races of H. vulgare: H. spontaneum, H. agriocrithon and H. lagunculiforme. Since these three races are grown and cultivated by man and since they are 100 percent sexually compatible with all domestic barleys, they do not qualify as separate species. The plant breeder who deals with barley has therefore had little reason to hope for variability produced through intergeneric or interspecific hybridization. Harlan (1966) has noted that such success is remote.

Despite such gainsaying, modest attempts and successes have been reported in wide hybrids of barley. In the attempt category Quinke (1940) published the first attempts of the cross H. vulgare (2n=2x=14) x H. jubatum (2n=4x=28). Morrison, et al. (1959), Vinogradova (1946, as cited in Smith, 1951; and Price, 1968) have also tried this cross, but sterility in the F1 hybrid has precluded further attempts. Bakhteiev and Darevskaya (1945) made the H. vulgare x Elymus arenarius and H. vulgare x E. giganteus crosses, each hybrid of which had 21 chromosomes. There have been no reports on any agronomic benefits as a result of these crosses.

Claims of successful gene transfers begin with Hamilton, et al. (1955) and Schooler (1967). Hamilton used H. leporinum and Schooler used H. bulbosum to transfer disease resisteance to cultivated barley. Additionally, Schooler (1967) and Ahokas (1975) have produced male sterile lines of barley from wide hybridization. And finally, Schooler (1979) transferred pubescence to barley and in the same paper reported that after years of screening in both the field and the greenhouse, resistance to net blotch (H. teres) had been successfully transferred from a complex cross. Beyond these modest successes nothing has been reported, lending credence to the bleak outlook expressed earlier by Harlan.

If this thesis accomplishes no other purpose than to demonstrate that imaginative plant breeding may yet crack the barriers to successful wide hybridization in barley, then the effort of these past years has been worthwhile. The research which follows details our attempts to circumvent the many problems which have so far impeded this necessary goal.

THE RESEARCH AND FINDINGS

Hybrids with H. jubatum

Hordeum jubatum (L.) (2n=4x=28) is a segmental allotetraploid (Starks and Tai, 1974). It is commonly known as squirrel tail barley and it is a perennial grassy weed throughout vast regions of the northern Great Plains and it extendseven into Alaska. The capacity of this grass to endure temperature extremes and to grow and proliferate in sodic soils, coupled with its genetic relatedness to domestic barley, highlighted H. jubatum as a likely donor of such traits to H. vulgare.

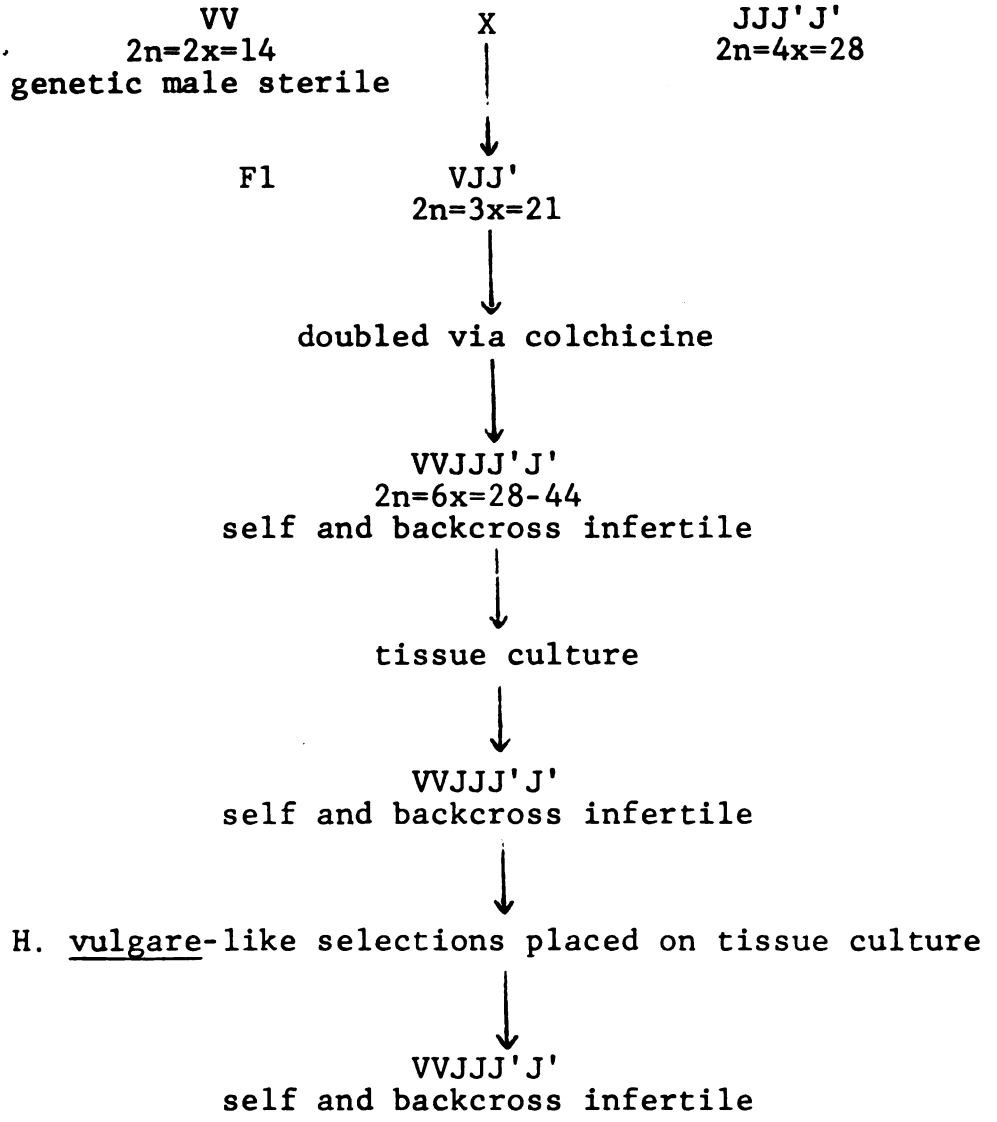
Material collected by Dr. John E. Grafius in Alaska was introduced into the breeding program at Michigan State University. Viable F1 progeny were finally produced by Steidl (1976) using a genetic male sterile H. vulgare as female. The seven F1 plants he produced were 2n=3x=21 and they were therefore sterile. This hybrid will hereafter be designated VJJ'. A cytogenetic study indicated a complete lack of homoeologous pairing between the chromosomes of H. vulgare and those of H. jubatum.

VVJJJ'J'

Attempts to restore the fertility of VJJ' by doubling

the chromosome complement via colchicine was without success (Steidl, 1976). The treated materia, VVJJJ'J', varied in chromosome number from 28 to 44 (Nelson, unpublished) yet produces no seed. Neither were backcross pollinations with H. vulgare successful: approximately eighty heads of VVJJJ'J' were pollinated and no embryos were formed. Due to the variability in both phenotype and chromosome number, it was hoped that a cycle of tissue culture induction and whole plant regeneration might enhance the variability by restoring some fertility through a further reduction in chromosome number. Twenty-three plants were regenerated and those plants which were capable of heading were pollinated. There were nineteen heads on eleven different plants pollinated with diploid H. vulgare and neither seed nor embryos formed on any of the plants. The heads which were not pollinated (approximately 20) did not set any seed.

Finally, another cycle of tissue culture was initiated using plants selected on a phenotypic basis from the previous tissue culture regenerates. The fifteen second-cycle plants headed and proved no more encouraging than those amphiploids from which they were derived. Therefore, further work with VVJJJ'J' has ceased. The following diagram outlines and summarizes the work to date on this hybrid.



Work Ceases

VJJ'

Introduction

The successful introgression of wild germplasm into any cultivated species has historically always required at least partial sexual compatability between the species. Within this context any further work with the H. vulgare -

H. jubatum hybrids for plant breeding purposes was pointless. The rediscovery by Orton (1979) of infrequent and spontaneous chromosome elimination in the F1 hybrid opened the door to further work with this hybrid.

The F1 hybrid, VJJ' (2n=3x=21) is in H. vulgare cytoplasm and Orton's and Steidl's discovery was of the spontaneous, directed elimination of the two H. jubatum genomes with the retention of the single, haploid H. vulgare genome (V: 2n=x=7). The implications are twofold and subtle. First, if any degree of somatic recombination has occurred between the chromosomes of the respective species then those haploid, chimeric sectors will contain some genetic material from H. jubatum. Secondly, any agent which can increase the frequency of somatic recombination will also increase the likelihood of recovering favorable genes from the H. jubatum genomes within the H. vulgare genome. Doubling the chromosome complement of the haploids then generates a population of plants whose value is proportional to the number of different incorporated H. jubatum genes as well as to the number of haploid plants originally isolated. Crucial to this scheme is the utilization of an efficient means to double chromosome numbers. Such a technique was worked out by Kasha (1978) and was subsequently used in this research.

The origin of these sectors was either directly from callus culture regeneration (four each) or from established F1 whole plants (thirteen each by June 1979). The common

element between these two origins is the fact that in both cases the sectors arose from triploid material which had gone through at least one cycle of tissue culture as opposed to Steidl's observation of sectoring in the original non-cultured hybrid which he produced. Those haploids which originated from whole plants occurred either singly or as a mass of tillers from an interface of V with VJJ' (Figure 1). Mitotic root tip chromosome counts of seventeen sectors confirmed that they were exclusively seven chromosome plants (Figure 2). There were no morphological indications of H. jubatum characteristics.

During autumn of 1978, a program was developed which would conceivably best exploit the potential of the isolated haploid sectors. A corollary program was developed which would utilize the sectoring phenomenon as well as enhancing the frequency of incorporation of H. jubatum genes within the single genome of H. vulgare.

The first program was the doubling of all isolated sectors to the diploid state. This would normally produce self-fertile plants, but since the original cross, VV x JJJ'J', was accomplished through the use of male sterile J. vulgare (genetic, single locus and recessive) the resulting diploid plants would be homozygous for the male sterile gene. Consequently, fertilization would have to be accomplished using another pollen source. This F1 seed would, in turn, be planted to produce F2 seed, which could be used for genetic analysis to determine the

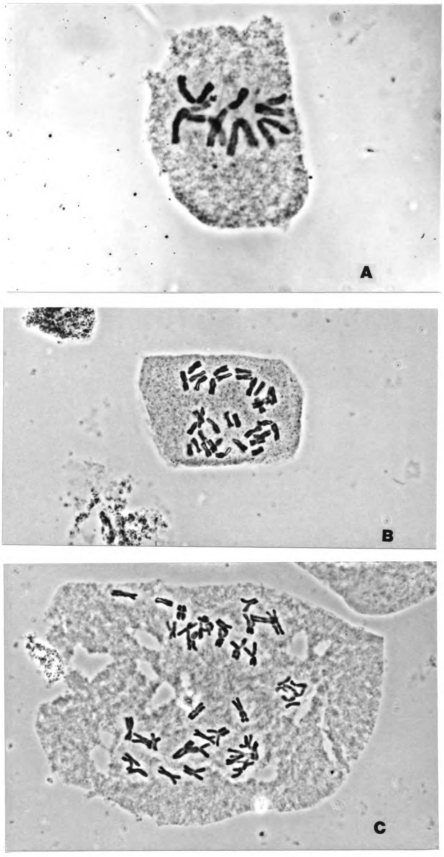
Figure 1. VJJ' (2n=3x=21) plant with haploid (2n=x=7) sectors emerging. Note the clasping auricles.

Figure 1

Figure 2. (A) Somatic metaphase of sector (2n=x=7) from VJJ' (2n=3x=21). (X1500)

(B)(C) Somatic metaphase of two sectors arising independently from AHV442 (after Huang) (2n=5x=35). (X1250)

Figure 2



possible presence of agronomically desirable genes transferred to H. vulgare from H. jubatum. The test chosen for this determination was that of Al-Shamma (1979) for seed germinability in solutions of NaCl.

Materials and Methods Involving Haploids

A total of fiteen haploid plants tillered sufficiently over a period of four months to produce between three and six culms of approximately three inches in length. Plants were divided at the crown with half being treated by Thiebaut, Kasha and Tsai's method (1979). The remaining plants were retained as reserve stock. Treated plants were grown and scrutinized for diploid morphology. Some plants which failed to diplodize were treated again. At flowering, the diploid plants were pollinated randomly with the commercial barley varieties Larker, Morex, Bonanza, Klages and Park. F1 seed from these crosses was collected over several days and dried at 100°F for one week to break secondary dormancy. The seed which was available by April 1979 was germinated in flats and transplanted to the field. Seed produced after April was planted in eight inch pots, placed for six weeks in a cold room and then grown to maturity in the greenhouse. The F1 plants grown in the field were treated once with Benlate to prevent powdery mildew.

F2 seed from the field and the greenhouse was harvested in July and September, respectively. Of fifteen original lines, nine survived in the field to produce

adequate seed for subsequent analysis. These seeds were stored until late November, 1979, when the germinability studies began.

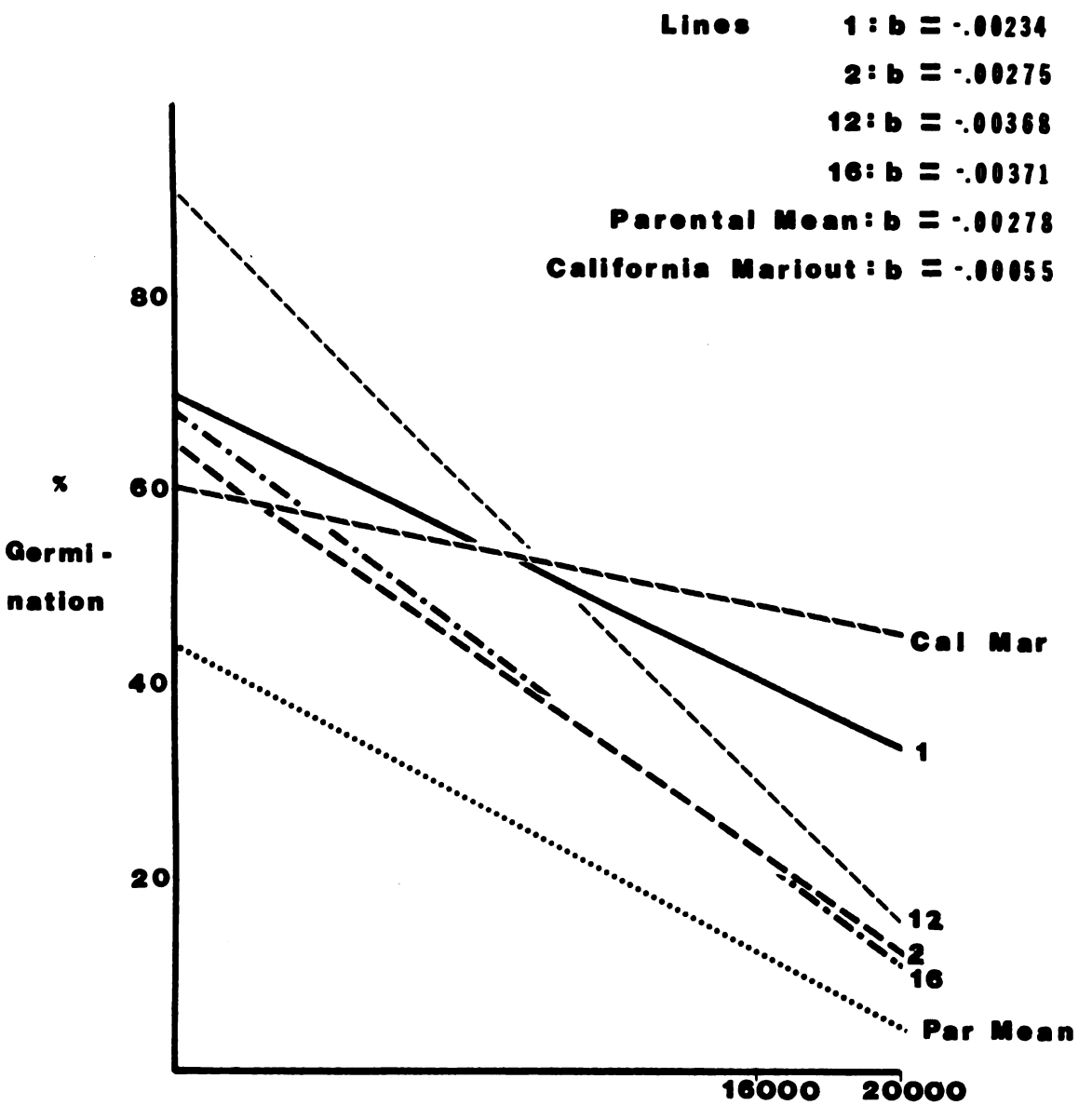
The seed used in the germinability experiment included California Mariout (a known salt-tolerant line), male sterile (used in the original cross which produced VJJ'), the five pollen parents listed earlier and the nine surviving lines produced in the field. The method used was a modification of Al-Shamma's technique in that smaller culture tubes were utilized and the temperature throughout the experiment was $24^{o}C$. Each culture tube contained 25 seeds and the solutions were distilled H_2O, 16×10^3 ppm NaCl and 20×10^3 ppm NaCl. The first experimental run contained two replications of three tubes each with 25 seeds per tube. Another two replicates were run through identical treatments again for a total of four replications of all nine lines, six parents and California Mariout.

Results and Discussion

The germination test showed that increasing concentrations of salt decreased the percentage germination in all entries, including the check variety California Mariout. The linear relationship between percentage germination and salt concentration is borne out by the regression analysis (Figure 3) for four selected experimental lines, the parental mean and California Mariout.

Figure 3. Regressions of four selected experimental lines and California Mariout for percentage germinability in three concentrations of NaCl.

FIGURE 3


Lines 1 : b = -.00234
2 : b = -.00275
12 : b = -.00368
16 : b = -.00371
Parental Mean : b = -.00278
California Mariout : b = -.00055
80
60
40
20
%
Germi-
nation
Cal Mar
1
12
2
16
Par Mean
16000
20000
ppm NaCl

As expected, California Mariout displayed clear superiority to both the line and the parents, and the experimental lines were superior to the parents. The regression analysis for the experimental lines alone indicate differences between them, with line 1 superior to all others.

Differences between all of the regression lines were calculated (Table 1 and 2) and showed highly significant differences between nearly every entry.

Superficial analysis of the data indicate that there exists a modest increase in percentage germination of the experimental lines over the maternal and paternal parents at both 16×10^3 and 20×10^3 ppm NaCl. If such differences do exist, then the only plausible explanation is that these differences are due to genetic transfer of genes involved in this trait from H. jubatum to H. vulgare. Furthermore, such transfer occurred solely in the somatic (non-sexual) tissue of the triploid hybrid VJJ'. This hypothesis is further supported by the differences found within the nine experimental lines. Each line was derived from individual haploid sectors on individual and distinct hybrid plants: hence, each haploid sector was an independent event with a chance of somatic recombination unrelated to the other haploids.

On the other hand, the superiority of the experimental lines may well be an example of drawing false conclusions from highly significant statistical results. The crux of the analysis rests upon the comparison of the

Table 1. Mean squares of salt tolerance scores as represented by germinating seeds of the nine experimental lines, the six parents and California Mariout.

Source of Variation	Degrees of Freedom	Mean Square	F
Block	3	9.42	
Entries	15	114.96	19.29***
Error (a)	45	5.96	
Treatment	2	3122.83	481.18***
Entries X Treatment	45	28.88	4.45***
Error (b)	81	6.49	

***$P \leq 0.005$

Table 2. t values for the difference between two slopes for the six varieties of barley for germination.

Entries	t
1 and	
2	3.41
3	14.97
4	8.73
6	14.80
7	11.89
12	11.14
14	2.66*
16	11.39
Parental mean	3.66
California Mariout	14.88
* $P \leq 0.01$; all others at $P \leq 0.001$	
2 and	
3	11.56
4	5.32
6	11.39
7	8.48
12	7.73
14	0.75*
16	7.98
Parental mean	0.25*
California Mariout	18.29
* Not significant; all others at $P \leq 0.001$	
3 and	
4	6.24
6	0.17*
7	3.08
12	3.82
14	12.30
16	3.58
Parental mean	11.31
California Mariout	29.85
* Not significant; all others at $P \leq 0.001$	
4 and	
6	6.07
7	3.16
12	2.41*
14	6.07
16	2.66@
Parental mean	5.07
California Mariout	23.61
* $P \leq 0.02$; @ $P \leq 0.01$; all others at $P \leq 0.001$	

Table 2. (Continued)

Entries	t
6 and	
7	2.91*
12	3.66
14	12.13
16	3.41
Parental mean	11.14
California Mariout	29.68
* $P \leq 0.01$; all others at $P \leq 0.001$	
7 and	
12	0.75*
14	9.23
16	0.50*
Parental mean	8.23
California Mariout	26.77
* Not significant; all others at $P \leq 0.001$	
12 and	
14	8.48
16	0.25*
Parental mean	7.48
California Mariout	26.02
* Not significant; all others at $P \leq 0.001$	
14 and	
16	8.73
Parental mean	1.00*
California Mariout	17.54
* Not significant; all others at $P \leq 0.001$	
16 and	
Parental mean	7.73
California Mariout	26.27
$P \leq 0.001$ for both	
Parental mean and	
California Mariout	18.54
$P \leq 0.001$	

experimental lines with the parents. In fact the parental seed used in the germination test was grown under separate environmental conditions. This is due to several reasons. The experimental lines were grown under severe time pressure in an attempt to produce F2 seed in the field. Since the male sterile maternal parent was also of winter habit, the F1 plants were germinated in the greenhouse and transplanted to the field in March in a desperate attempt to grow them under a sufficiently cold spring environment to vernalize them. Without vernalization the plants would never head and there would be no F2 seed for analysis. The mortality of these lines in the field bear this out; only nine lines survived to produce adequate seed for the germination test. The pollen parents were, however, exclusively spring varieties and they were planted in mid-April and they thrived.

The germination tests were therefore performed on seeds of differing vigor. Ordinarily one would conclude that the parental varieties should have outperformed the experimental lines, especially at 0 ppm NaCl. The opposite was the case. The mean parental germination percentage under this treatment was about half that of the lines. This could be attributed to anaerobic degradation of the parental seed in the solutions. This effect did not show up in the other lines since the quantity of their endosperm was but a fraction of the parents. The experimental

germinated well despite their poor quality and I did not have adequate seed to perform another trial.

The question of true superiority should be answered within a year since these are the only lines with which I have to work. If there has been some somatic recombination then those genes which were transferred and which confer greater salt tolerance are probably located at random throughout the genome of the experimental lines. This clearly leads to the possibility that hybridization between these lines will result in F2 transgressive segregants for the trait in question. A 4x4 diallel cross of the four most promising lines which germinated in the $20x10^3$ ppm NaCl solution will be made. Subsequent comparison of the F2 progeny means with the means of the parents should either confirm or refute the hypothesis that these lines bear genes for the salt germinability character. Furthermore, crossing the superior lines into California Mariout and then backcrossing to California Mariout may even improve its already high germinability in $20x10^3$ ppm NaCl. Production of F2 seed in the field should also be facilitated since all putative parents are now segregating for the spring character.

The second program involving VJJ' was (and is) a series of treatments *in vitro* to either enhance somatic recombination or to produce translocations between the H. *vulgare* and H. *jubatum* genomes. Regenerated plants from these treatments might then be expected to generate

haploid sectors which would presumably contain within the H. vulgare genome genes transferred from H. jubatum.

Materials and Methods Involving Treatments

Immature inflorescences from VJJ' were placed on maintenance and inducation medium. Primary callus of one to three months of age were treated with 10^{-5} and 10^{-6} M mitomycin-C and 5-bromodeoxyuridine (BuDR). Additionally, primary callus was treated with gamma radiation from a Cobalt-60 source at the approximate dosage of 2 kiloroentgens in an effort to produce chromosomal translocations.

The two chemical were incorporated into freshly prepared maintenance and inducation medium which had cooled sufficiently for the container to be handled without gloves. In the case of the BuDR, the procedures (including preparation) were done in near-darkness. Primary callus was placed on the medium for at least several weeks--a period sufficient for two cell cycles. After culturing, the calli were transferred to regeneration medium and eventually whole plants were recovered.

Irradiation of callus material presented some special problems, primarily those of contamination. Since cultures were irradiated in petri plates the petri plates were suspended vertically, or orthogonal to the path of the radiation. Whenever enough condensation had accumulated inside the plate on the surface of the lid, the risk of contamination was always high. When suspended,

the condensed water runs to the bottom of the plate and usually to the outside where it serves as a wick for bacterial and fungal contamination. Choosing only those plates which had little or no condensation reduced the contamination. After irradiation all calli were transferred to fresh medium to avoid the toxic effects of free radicals in the irradiated medium.

Results and Discussion

Sixty-three plants treated with 10^{-5} M mitomycin-C have been regenerated from callus. None have produced haploid sectors. The BuDR and irradiated materials are currently being produced and regenerated. Seven plants from callus treated with 10^{-5} M BuDR are growing in the greenhouse. No haploid sectors on these plants have yet formed. Now that the procedures have been constructed, the sole objects of this phase of research is to build the populations of treated VJJ' plants and then wait for haploid sectors to form.

When and if promising sectors do arise there are several additional means by which gene transfer can be determined, aside from running a test for germinability in salt solution. The first is the classic cytological F1 hybrid test. Substantial genetic incorporation or translocations can be expected to show up at metaphase and anaphase I through abnormal pairing and migration to the poles. The second method would be the use of

polyacrylamide gel electrophoresis. The most likely protein for this assay have yet to be determined.

AHV

The numerous Agropyron - Hordeum (AHV) hybrids produced by Rye-Ho Huang (1976, 1978) were an attempt to circumvent the sterility barriers found in the H. vulgare - H. jubatum hybrids. The goal in breeding this new complex of hybrids was to generate genetic variability for agronomically desirable traits in barley. Only the more promising hybrids used in this research will be reported. The notation for the hybrids is that of Huang.

In winter of 1978 hundreds of spikes of SHV-444 were emasculated and pollinated with diploid H. vulgare. No seeds and no embryos were produced.

The incorporation of more hybrids by 1979 into my program greatly expanded the opportunities. Foremost among these hybrids was AHV-444 (Huang, 1978) which, due to partial fertility, produced eleven seeds when emasculated and then pollinated with a random mixture of spring barley varieties. From these eleven seeds eight plants were produced through embryo culture. It was discovered that this cross had already been produced by Huang, but with one difference: Huang used an autotetraploid instead of a diploid barley as the pollen source. Huang designated this cross AHV-B and I designated the diploid crosses as AHV-B-N. Eventually the AHV-442 produced 144

seeds by selfing, the plants from which were morphologically dissimilar to both AHV-B and AHV-B-N.

At anthesis second backcrosses of AHV-B and AHV-B-N to H. vulgare (2n=2x=14) were attempted. More than one hundred spikes were pollinated without a single seed or embryo developing. Thus ended another futile series of pollinations and months of effort.

The haploid chimeric secoring phenomenon which occurred in VJJ' now presented itself as a possible means by which to effect both a chromosome reduction in the Agropyron hybrids and a restoration of at least partial fertility. Consequently a cycle of tissue culture induction and regeneration was initiated in hopes of stimulating genomic instability in the hybrids. Existing callus was regenerated and more callus was irradiated with Cobalt-60 at a dosage of 2-3 x 10^3 Roentgens. The callus was then placed on a regeneration medium. Additionally callus was treated with 10^{-5} M BuDR, grown in the dark while on a medium containing BuDR for three weeks. After exposure to light the calli were transferred to regenration medium.

Surviving plants of the two treatments were transplanted to the greenhouse. After nine months of vigorous growth neither of the AHV-B groups had produced sectors. All the plants are morphologically indistinguishable from each other. Of the hundreds of heads these plants have produced, none has set any seed and none has backcross fertility to H. vulgare.

The first evidence of spontaneous chromosome elimination in the Agropyron - Hordeum hybrids occurred in Autumn, 1979. Sectors from AHV-444 and from AHV-B had arisen and were distinguished from their progenitors by possessing vestigial--but pronouned--clasping auricles, a morphological genetic marker in barley. Mitotic root tip counts of these two sectors confirmed that a chromosome reduction had occurred in AHV-444. This hybrid is composed of Agropyron trachycaulum, Hordeum jubatum and H. vulgare such that its somatic chromosome number is 42. The chromosome counts on this sector showed 35--a loss of one complete genome (Figure 4). The parentage of the hybrid AHV-444 and its sector follows (after Huang):

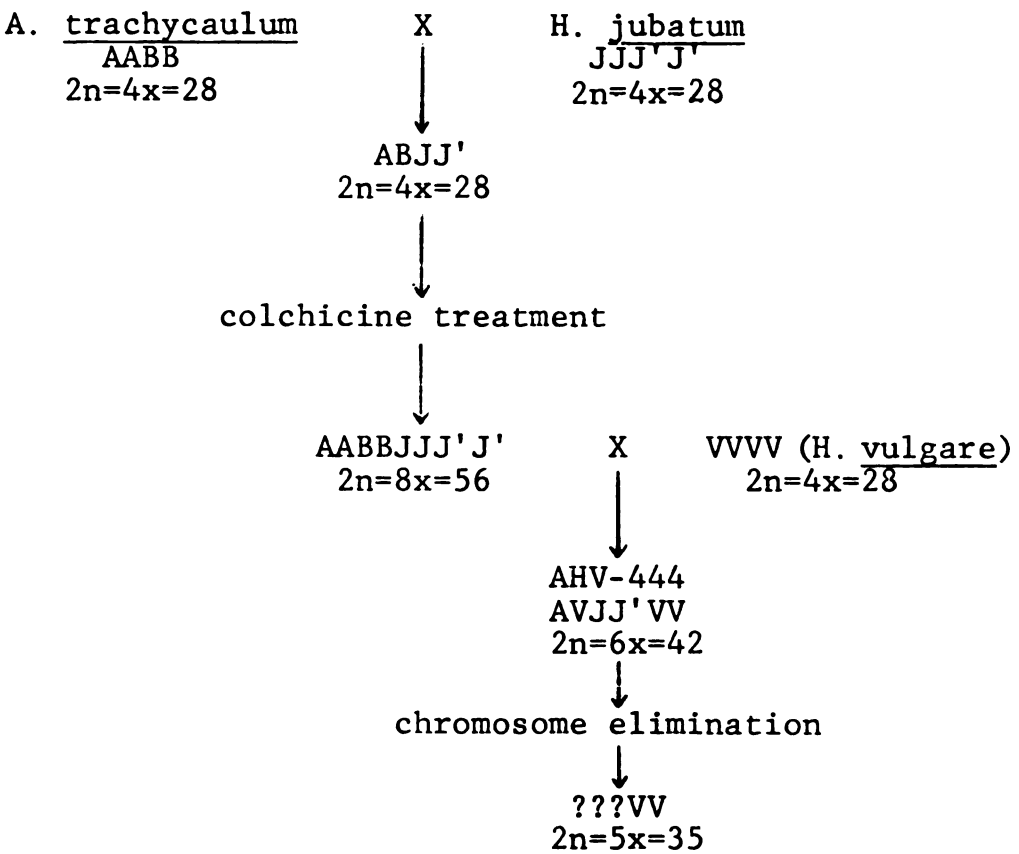
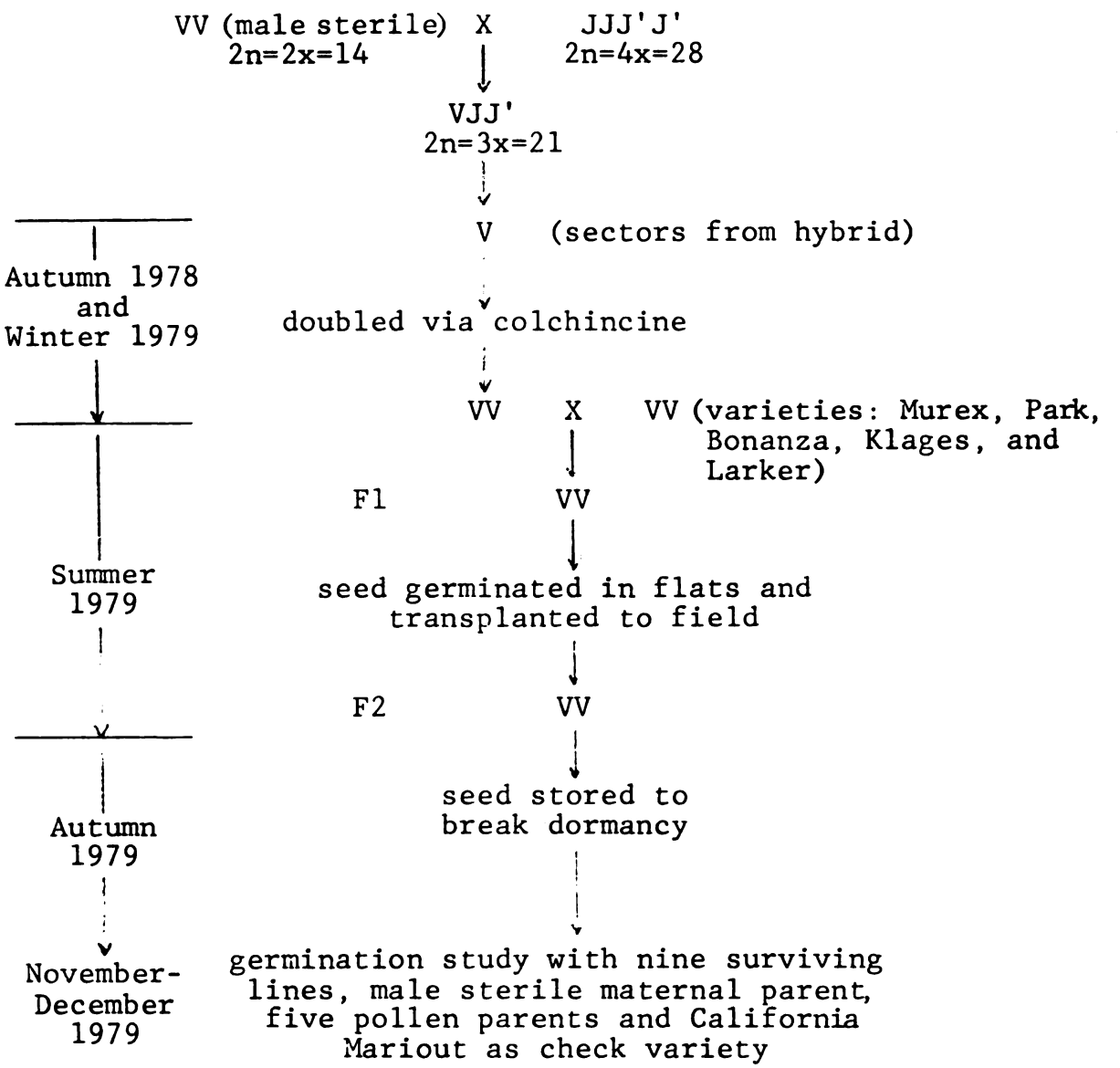
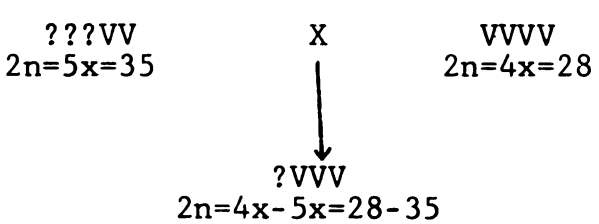


Figure 4. Schedule of dihaploid seed production and screening for germinability in three solutions of varying concentrations of NaCl.



The second sector originated from a plant labelled AHV-B. Since division and increase, none of these clones is morphologically distinguishable from the AHV-444 sector and the chromosome count of 35 (Figure 2) strongly implies that the hybrid from which it arose was not AHV-B, but that the parent was WHV-444 instead. Considering also that AHV-B has but 35 chromosomes, any somatic reduction in its number will necessarily be fewer than the 35 found in the sector also suggest that the pot was simply mislabeled.

The two sectors were divided and propagated vegetatively. At flowering they were pollinated with H. vulgare. A total of 41 spikes have been pollinated with no seed set or embryo formation. Considering the high chromosome number this is not surprising. No selfed seed has formed either. Immature inflorescences from these sectors have been placed on medium for future treatment and approximately 10^3 anthers have been placed on potato medium culture (Schaeffer, et al., 1979) adjusted to pH = 6.0. Thought has been given to the use of autotetraploid H. vulgare as a pollen parent. The putative cross would be as follows:



The success of such a cross is indeed speculative.

BIBLIOGRAPHY

BIBLIOGRAPHY

Al-Shamma, A. M. 1979. A genetic study of salt tolerance in barley (H. vulgare L.). M.S. Thesis, Michigan State University.

Bakhteiev, F. and E. M. Darevskaya. 1945. An intergeneric hybrid between barley and Elymus. Barley Genetics Newsletter, Vol 5.

Cooper, K. V. 1978. Hybrid plants from the barley X rye cross. Plant Sci. L. 12 (3/4): 293-298.

Hamilton, D. G., S. Symko and J. W. Morrison. 1955. An anomalous cross between Hordeum leporinum and Hordeum vulgare. Can. J. Agr. Sci. 35: 287-293.

Harlan, J. R. 1966. Plant introduction and biosystematic. In: Plant Breeding: A Symposium held at Iowa State University. Ed. K. J. Frey. Iowa State U. Press, Ames, Iowa: 55-75.

Huang, Rye-Ho. 1978. Intergeneric hybridization in crosses involving barley and its wild relatives. Ph.D. Dissertation, Michigan State University.

Morrison, J. W., A. E. Hannah, R. Loiselle, and S. Symko. 1959. Cytogenetic studies in the genus Hordeum. Can. J. Plant Sci. 39: 375-383.

Orton, T. J. 1978. A study of regeneration and karyotypic variability in tissue cultures of H. vulgare, H. jubatum and their interspecific hybrid. Ph.D. Dissertation, Michigan State University.

Orton, T. J. 1979. Quantitative analysis of growth and regeneration from tissue cultures of Hordeum vulgare, H. jubatum and their interspecific hybrid. Envir. Exper. Bot. 19: 319-336.

Price, P. B. 1968. Interspecific and intergeneric crosses of barley. In: Barley: Origin, Botany, Culture, Winterhardiness, Genetics, Utilization and Pests. Ag. Handbook No. 338, ARS-USDA: 85-95.

Quinke, F. L. 1940. Interspecific and intergeneric crosses with Hordeum. Can. J. Res. C 18: 372-373.

Schaeffer, G. W., P. S. Baenziger, and J. Worley. 1979. Haploid plant development from anthers and in vitro embryo culture of wheat. Crop Sci. 19: 697-702.

Schooler, A. B. 1964. Wild barley crosses show disease resistence. N. Dakota Farm Research 23: 13-15.

Schooler, A. B. 1967. A form of male sterility in barley. J. Hered. 58: 206-211.

Schooler, A. B., and M. K. Anderson. 1979. Interspecific hybrids between (Hordeum brachyantherum L. x H. bogdanii Wilensky) x H. vulgare L. J. Hered. 70: 70-72.

Smith, L. 1951. Cytology and genetics of barley. Bot. Rev. 17: 1-355.

Starks, G. D., and W. Tai. 1974. Genome analysis of Hordeum jubatum and H. compressum. Can. J. Genet. Cytol. 16: 663-668.

Steidl, R. P. 1976. Hybridization of barley (H. vulgare L. Emend. Lam) with its wild relatives. Ph.D. Dissertation, Michigan State University.

Thiebaut, J., K. J. Kasha, and A. Tsai. 1979. Influence of plant development stage, temperature, and plant hormones on chromosome doubling of barley haploids using colchicine. Can. J. Bot. 57: 480-483.

Vinogradova, N. M. 1946. Hybridization between cultivated barley and wild species of Hordeum. Trudy Zonal. Inst. Zerhavova Khoziaistva Nechernozemnoi Polosy SSR 13: 134-137.

Whitmore, E. T., and D. H. B. Sparrow. 1957. Laboratory micromalting technique. J. Inst. Brew. 63: 397-398.